

WHAT IS CLAIMED IS:

1 1. A method for purification, modification and immobilization of recombinant protein,
2 said method comprising the steps of:
3 tagging a DNA sequence encoding a target protein into a recombinant vector with
4 a specific tag sequence;
5 expressing the vector under suitable condition to obtain a recombinant protein;
6 purifying and modifying said recombinant protein by using an affinity column and
7 a modification reagent;
8 exchanging said recombinant protein which has been attached to the affinity
9 column with a decoupling reagent; and
10 immobilizing said recombinant protein onto a substrate.

1 2. The method as claimed in claim 1, wherein said specific tag comprises Histidine
2 tag, Maltose-binding tag, or GST tag.

1 3. The method as claimed in claim 2, wherein said specific genetic tag is Histidine
2 tag.

1 4. The method as claimed in claim 1, wherein said recombinant protein is prepared
2 by using prokaryotic cell, eukaryotic cell or an *in vitro* transcription/translation system.

3 5. The method as claimed in claim 4, wherein said prokaryotic cell is *E. coli*.

4 6. The method as claimed in claim 4, wherein said eukaryotic cell is yeast, insect
5 cell or mammalian cell.

6 7. The method as claimed in claim 1, wherein the affinity column for capturing the
7 recombinant protein is chosen in corresponding to said specific tag.

1 8. The method as claimed in claim 7, when said specific tag is Histidine tag, a metal
2 chelating column is used as the affinity column.

1 9. The method as claimed in claim 8, wherein the metal chelation column is
2 represented by a general formula as metal-X column.

1 10. The method as claimed in claim 9, wherein the metal in said formula comprises
2 nickel, zinc, copper, or cobalt.

1 11. The method as claimed in claim 9, wherein the X in said formula comprises
2 iminodiacetic acid, nitrilotriacetic acid, tris(carboxymethyl)ethylendiamin,
3 carboxymethylaspartate, or TALON.

1 12. The method as claimed in claim 9, wherein the metal-X column is Ni-IDA
2 column or Cu-IDA column.

1 13. The method as claimed in claim 7, when said specific tag is Maltose-binding tag,
2 an amylose column is used as the affinity column.

1 14. The method as claimed in claim 7, when said specific tag is a GST-tag,
2 glutathione column is used as the affinity column.

1 15. The method as claimed in claim 1, wherein said recombinant protein is
2 modified by using a biotinylation reaction so to add biotin functional groups to said
3 recombinant protein.

1 16. The method as claimed in claim 15, wherein the modification of said
2 recombinant protein comprising the steps of:

3 obtaining a solution containing the recombinant protein;

4 adding a biotinlyation reagent to cause biotinlyation reaction with said recombinant
5 protein; and

6 capturing said biotinlyted recombinant protein by using the affinity column so as to
7 fixate said biotinlyted recombinant protein in said affinity column.

1 17. The method as claimed in claim 15, wherein the modification of said
2 recombinant protein comprising the steps of:

3 obtaining a solution containing the recombinant protein;

4 capturing said recombinant protein by using the affinity coloum so as to fixate said
5 recombinant protein in said affinity column; and

6 adding a biotinylation reagent to said affinity column to cause biotinylation
7 reaction with said recombinant protein fixated in said affinity column.

1 18. The method as claimed in claim 16, wherein said recombinant protein is
2 exchanged from the affinity column by a decoupling reagent, said decoupling reagent is
3 chosen according to the properties of the specific tag and the affinity column.

1 19. The method as claimed in claim 18, when said specific tag is Histidine tag and
2 the affinity column is a metal chelating column, the decoupling reagent is immidazole.

1 20. The method as claimed in claim 18, when said specific tag is maltose-binding
2 tag and the affinity column is an amylose column, the decoupling reagent is maltose.

1 21. The method as claimed in claim 18, when said specific tag is GST tag and the
2 affinity column is a glutathione column, the decoupling reagent is glutathione.

1 22. The method as claimed in claim 1, wherein the immobilization of said
2 recombinant protein is achieved by modifying the recombinant protein with biotin and
3 attaching the biotin-modified recombinant protien on a substate coated with streptavidin.

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